PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application	:	Emiliano Ghinelli
Application No.	:	10/665,188
Filed	:	September 17, 2003
Confirmation No.	:	5560
For	:	HUMAN AMNIOTIC MEMBRANE EXTRACT COMPOSITION FOR PROPHYLAXIS AND TREATMENT OF DISEASES AND CONDITIONS OF THE EYE AND SKIN
Examiner	:	Taeyoon Kim
Attorney's Docket	:	EMIL-001XX

TC Art Unit: 1651

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I hereby certify that this correspondence is being sent via facsimile to Examiner Taeyoon Kim, TC Art Unit 1651, Fax No. (571) 273 8300, on Sept. 4, 2007.

By: Holliday C. Heine
Holliday C. Heine, Ph.D.
Registration No. 34,346
Attorney for Applicant(s)

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DECLARATION OF EMILIANO GHINELLI, M.D. UNDER §1.132

VIA FACSIMILE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Emiliano Ghinelli declare:

1. I am a Medical Doctor specialized in Ophthalmology, holding at the present time the position of Director of Ophthalmology Service in two Hospitals: (1) OSPEDALE CIVILE DI VOLTA MANTOVANA s.r.l. Via Tonello, 5 - Volta Mantovana (MN), Italy; and (2) OSPEDALE S. PELLEGRINO DI CASTIGLIONE DELLE STIVIERE s.r.l. Via Garibaldi - Castiglione delle Stiviere (MN), Italy.

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2. I am the inventor of the invention described and claimed in the above-identified patent application.

3. I have read and understood the Final Office Action of the Examiner dated June 1, 2007, rejecting the currently pending claims 10-12 and 20-24 for obviousness over Kim et al. (KR2001098716A), alone or in combination with Carlsson et al. (US 6,117,857).

4. The invention described and claimed in the instant application is directed to a novel formulation for the therapeutic components of amniotic membrane, e.g., human amniotic membrane, a pharmaceutical composition that includes a therapeutically effective amount of an amniotic membrane extract preparation (AMX) consisting essentially of a powdered form of a lyophilized amniotic membrane homogenate supernatant, which can be reconstituted in a pharmaceutically acceptable carrier and wherein the mammalian amniotic membrane was subjected to only one freezing step during preparation of the extract.

5. As it is based on an extract prepared from a homogenate supernatant, the novel amniotic membrane formulation of the invention has been rid of cellular and intracellular debris. Yet, as the mammalian amniotic membrane was subjected also to only one freezing step during preparation of my extract, all of the important therapeutic factors determined by others to be present in an amniotic membrane are also present in the

formulation of the invention. These factors can not only be detected but also quantified. Furthermore, as AMX is a homogeneous powder, the extract can be reconstituted in a pharmaceutically acceptable carrier at the concentration desired for a particular application, e.g., as in the original membrane or several times more concentrated than the original membrane to treat diseases not treatable by others using previously known amniotic membrane preparations. Thus, the amniotic membrane extract formulation according to my invention has the healing properties of amniotic membrane tissue, but at an enhanced level, and can be used as described in the instant application without the need for costly surgery.

6. In the Final Office Action, the Examiner has stated that currently pending claims 10-12 and 20-24 are obvious over a newly cited Korean Patent Application to Kim et al. (KR2001098716A), alone or in combination with Carlsson et al. (US 6,117,857).

7. The Examiner characterizes Kim et al. as follows:

> Kim et al. teach a pharmaceutical composition comprising human amniotic membrane extract made by the process steps of 1) freeze-drying (lyophilized) and pulverizing (powdered) amniotic membrane and 2) homogenizing the powdered amniotic membrane, followed by centrifugation to obtain homogenate supernatant (see p.3, paragraph 4 of translated version). Reconstitution step of the claimed invention would be inherently carried out in Kim et al's method step because the powdered amniotic membrane has to be reconstituted in a solution for homogenization and centrifugation.

8. In my opinion as one of skill in the use of amniotic membrane preparations, the products described in Kim et al. are not at all like the products claimed in my application. I believe that the Examiner has misunderstood the process taught in the Kim et al. application and, therefore, has not appreciated the major differences between the Kim et al. products and those of my invention.

9. Further to my statements in my earlier Declaration, where I pointed out that my novel formulation is "a pharmaceutical composition that includes a therapeutically effective amount of an amniotic membrane *extract* preparation (AMX) consisting essentially of a *powdered form* of a *lyophilized* amniotic membrane *homogenate supernatant reconstituted* in a pharmaceutically acceptable carrier," I would now like to point out that my method of preparing my extract preparation *includes only one freezing step*. I will next explain the importance of this statement.

10. The mammalian amniotic membrane (amnion) is well-known for showing powerful and interesting healing properties and for containing a long list of therapeutically important factors. Processing the amnion for therapeutic use so that the critical healing factors are preserved has been problematic. I have determined, however, that an active and stable amniotic membrane extract *can* be prepared if the tissue is processed quickly and with care and is subjected to only one freezing step.

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Mammalian tissue isolated from the mammalian body immediately starts a process of autolysis as cell degradation begins and proteolytic enzymes are released. The longer the time taken for the preparative procedure, the longer is the exposure to the autolysis process. In addition, many thermal jumps or changes in temperature, either cooling or warming jumps, accelerate these destructive processes. I am well-aware of these issues and so developed my preparative procedure accordingly.

Referring now to Example I of my application, pp. 11-12, it can be seen therein that from the time of removal of the amniotic membrane from the pregnant woman who has just been delivered of her baby, through the homogenization and centrifugation steps, all procedures in my extraction process are carried out at approx. 4 °C (i.e., there is no freezing step). It is not until my homogenate supernatant is collected and divided into aliquots that it is quickly frozen (the only freezing step) and then kept in the frozen state until the extract material is "lyophilized," which means that the extract is maintained in its frozen state (in a small aliquot or in a frozen shell on the inside of a lyophilization bottle) while being evaporated to dryness under vacuum. The product of this process is a "powder" (see p. 12, line 14) which can be stored essentially indefinitely before being reconstituted for a specific use.

I emphasize that there is only one freezing step in this procedure and that my preparation is never thawed after that freezing step until the extract powder is produced. My

preparation procedure protects important factors in the amnion in sufficient amount that their concentrations can be quantified.

11. The process of Kim et al., as indicated at the place analyzed by the Examiner (p. 3, paragraph 4 of the translated version), is very different. After isolation and washing, the Kim et al. amnion was placed in a stock solution, frozen (for the *first freezing step*) and "dried." This could not mean "dried" as in "freed from liquid," however, as in the next series of steps, the frozen amnion is "pulverized" using a mortar (and, presumably, a pestle), an activity that will substantially raise the temperature of the amnion - by the way; "homogenized"; and then "centrifuged" (for a very long time at a low speed) with the "supernatant" being isolated, which means liquid was present, even if frozen, when the amnion material was "pulverized." The collection of a supernatant means that the material being processed *must* be liquid at this point, and this liquid is the original "stock solution." The Examiner is in error when he states that this could be considered a "reconstitution step."

The last lines of this paragraph indicate that the isolated and filtered Kim et al. supernatant is the material used for treatment without further "reconstitution." It is indicated earlier in the application (at p. 2, third paragraph from the bottom, last sentence of the translated version) that the filtered amniotic extract also can be "dried," which probably means freeze-dried, and pulverized. This would be the *second*

freezing step in the Kim et al. process, and, thus, if carried out, would expose the Kim et al. extract to an *extra period of self-destruction time* (autolysis) compared to my preparative method in order to reach a "powdered" form.

12. The Kim et al. product has not been analyzed for specific protein content. Referring to the top of p. 2 in the translated version, the list of drawings recites no protein analysis steps. Proteins that *might* be present in the Kim et al. extract are discussed in the 2nd paragraph on p. 3 of the translation. However, this discussion relates to the amnion *before* the Kim et al. processing steps and is *only theoretical* in regard to the extract itself. It is my belief that, given the Kim et al. processing steps, it is highly unlikely that specific proteins *could* be quantified in this "extract" in a repeatable manner.

In contrast, as mentioned above, I have been able to quantify the concentration of a number of specific factors in my reconstituted extract. I have attached hereto the results of assays recently carried out on samples of my amniotic membrane extract (AMX) prepared as described in the instant application. Western blot analysis revealed proteins having an apparent MW consistent with the presence of fibronectin, NGF, BDNF and NT-3. ELISA tests specifically detected NGF levels at 8.4 pg/mL, TGF-α levels at 15.4 pg/mL, NT-3 levels at 25.05 pg/mL and IL-1ra levels at 851.11 pg/mL.

13. Therefore, given that, as I have shown, I have produced a product in which the important therapeutic factors of the amnion

not only are still present but also can be quantified and, thus, administered to a patient in an amount appropriate for a specific therapy and given that Kim et al. have shown no ability to quantify any therapeutic factors that might remain in their product, it is my opinion as one of skill in the art that the "extract" disclosed in Kim et al. is completely different from my amniotic membrane extract (AMX), and that the teachings of Kim et al., either alone or in combination with other references, cannot make obvious my invention as claimed in the instant application.

I hereby declare that all statements made herein on personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this _____ day of _____, 2007.

_____

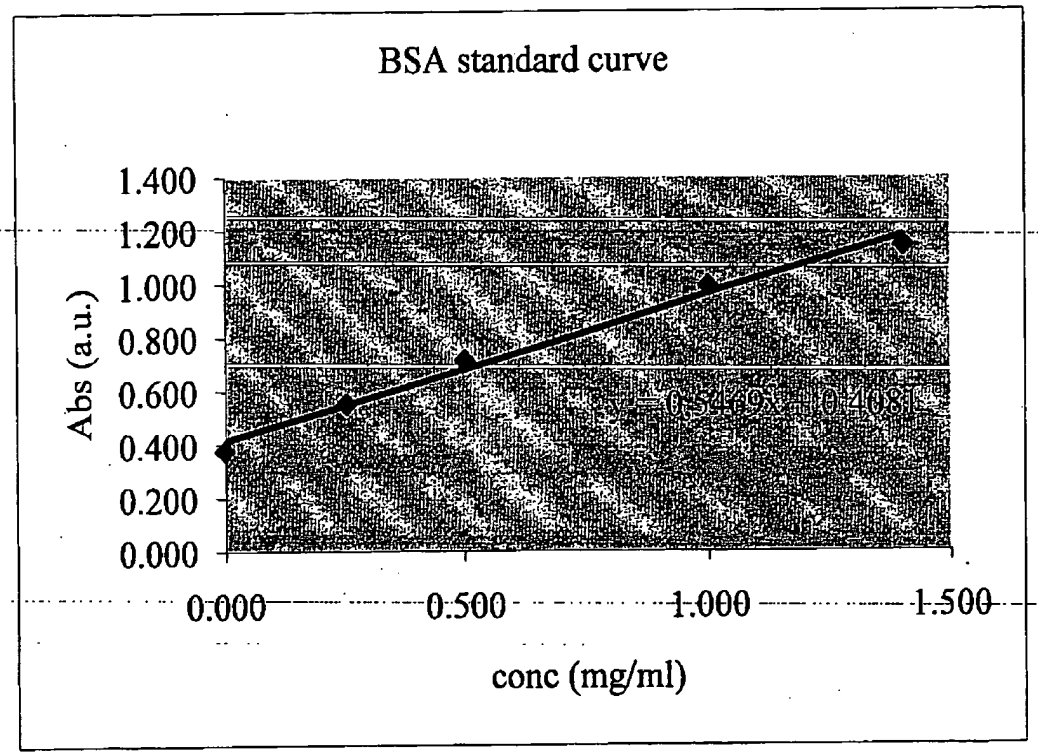
Emiliano Ghinelli

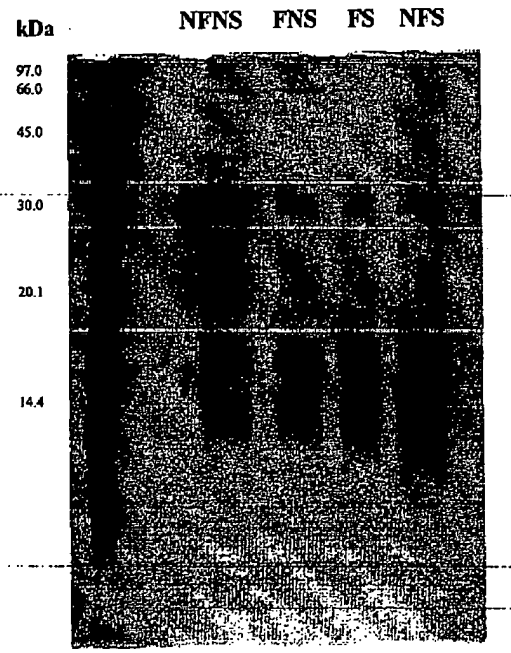
HCH/
356293.1

Appl. No. 10/665,188 – Attachment to Ghirelli Declaration of Sept. 4, 2007

MOLECULAR STUDIES:

AMX TOTAL PROTEIN CONCENTRATION





BSA conc mg/ml	Abs
0,000	0,371
0,250	0,555
0,500	0,711
1,000	0,989

SAMPLE	mean concentration (mg/ml)	weighted mg	resuspension volume (µl)	µg protein/mg weighted	% of recovery
NF NS	0,209 ± 0,002	1,5 ± 0,1	160 ± 1	22,280	100
F NS	0,119 ± 0,004	1,2 ± 0,1	160 ± 1	12,658	56,8
F S	0,091 ± 0,010	1,3 ± 0,1	160 ± 1	9,732	43,7
NF S	0,136 ± 0,013	1,7 ± 0,1	160 ± 1	14,543	65,3

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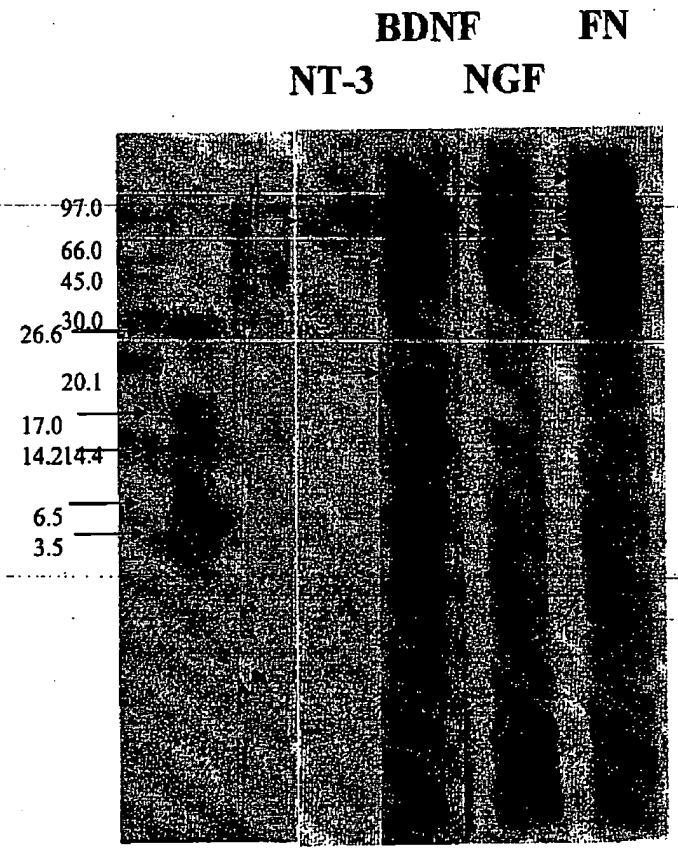
MOLECULAR STUDIES:

WESTERN BLOT



Apparent MW consistent for :

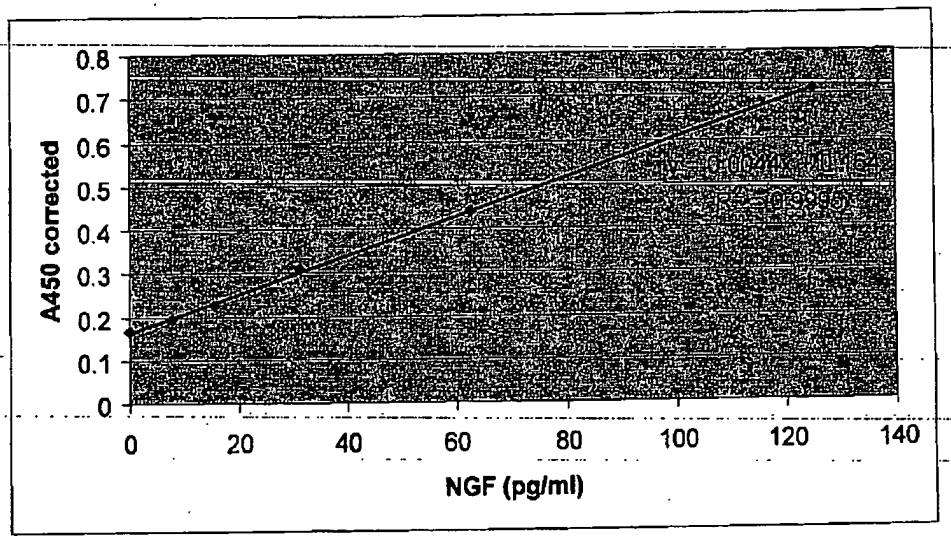
Fibronectin, NGF, BDNF and NT-3 presence

MOLECULAR STUDIES:

ELISA TEST (Promega NGF Emax)

Standard Curve

pg/ml				Mean	Std. Dev.
500	1.612	1.564	1.522	1.566	0.045033
250	1.157	1.043	1.04	1.08	0.066701
125	0.771	0.683	0.686	0.713333	0.049963
62.5	0.435	0.442	0.442	0.439667	0.004041
31.3	0.314	0.315	0.298	0.309	0.009539
15.6	0.219	0.225	0.237	0.227	0.009165
7.8	0.19	0.192	0.203	0.195	0.007
0	0.149	0.157	0.195	0.167	0.024576



NGF levels: 8.4 pg/mL

$p < 0.01$

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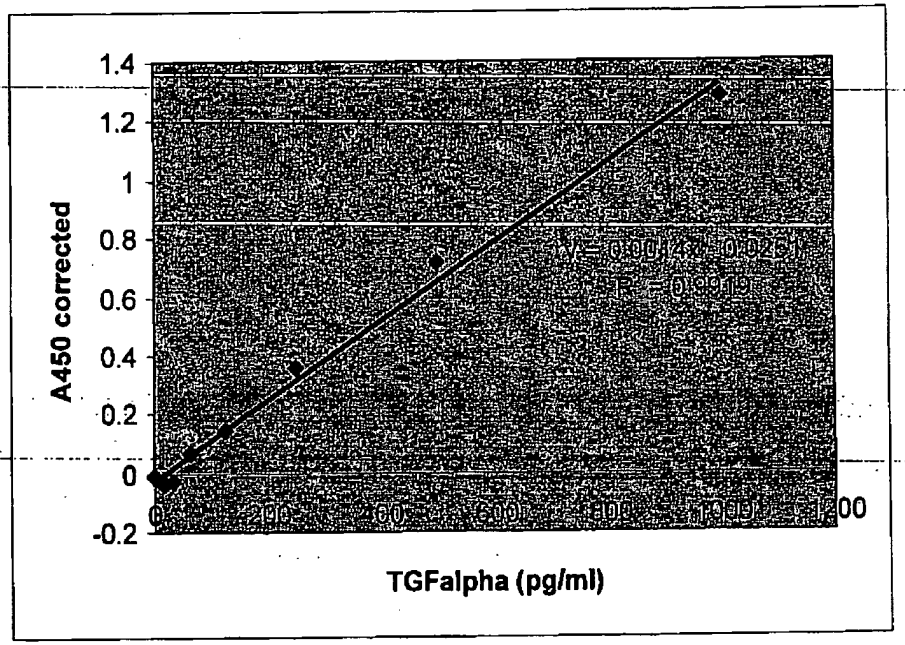
MOLECULAR STUDIES:

ELISA TEST (R&D TGF-alpha Quantikine)

Standard Curve

pg/ml				Mean
1000	1.164	1.286	1.402	**1.284**
500	0.708	0.637	0.812	**0.719**
250	0.297	0.398	0.376	**0.357**
125	0.144	0.082	0.196	**0.140667**
62.5	0.056	0.12	0.012	**0.062667**
31.2	-0.064	0.04	-0.072	**-0.032**
15.6	-0.051	0.026	-0.1	**-0.04167**
0	-0.081	-0.007	0.065	**-0.00767**



TGF-alpha levels: 15.4 pg/mL

$p < 0.01$

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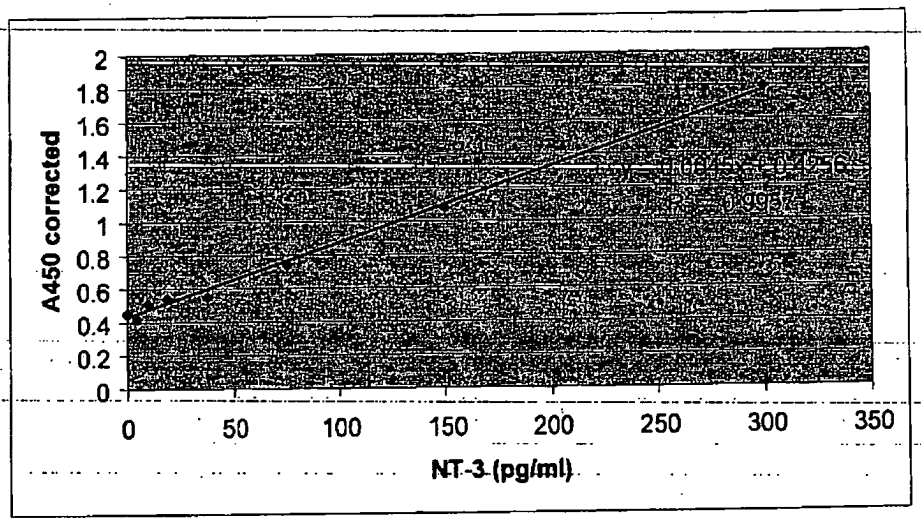
MOLECULAR STUDIES:

ELISA TEST (Promega NT-3 Emax)

Standard Curve

pg/ml				Mean	Std. Dev.
300	1.728	1.992	1.657	1.792333	0.176523
150	1.113	1.086	1.038	1.079	0.037987
75	0.743	0.737	0.739	0.739667	0.003055
37.5	0.567	0.544	0.553	0.554667	0.01159
18.8	0.54	0.485	0.599	0.541333	0.057012
9.4	0.499	0.461	0.561	0.507	0.050478
4.7	0.391	0.421	0.447	0.419667	0.028024
0	0.403	0.513	0.426	0.447333	0.05802



NT-3 levels: 25.05 pg/mL

$p < 0.01$

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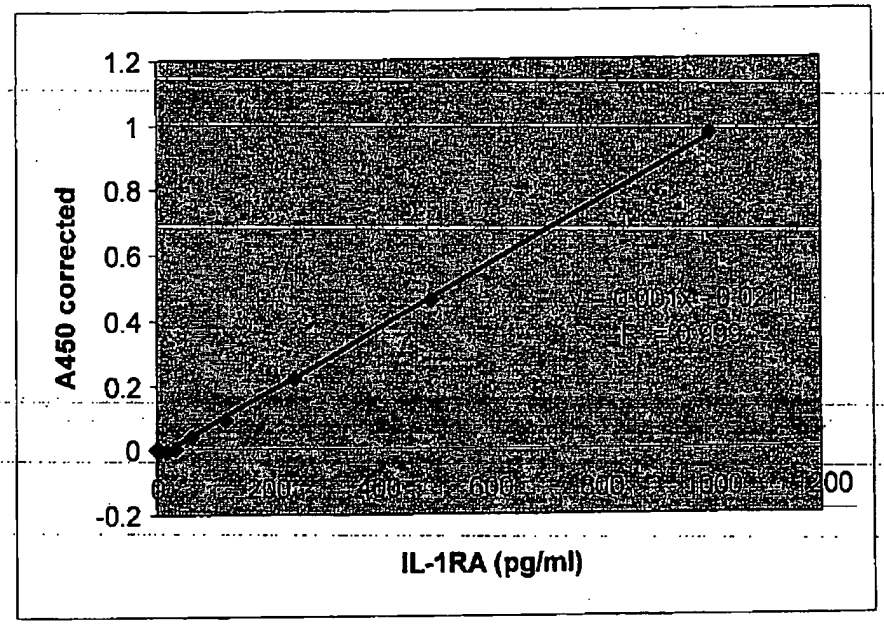
MOLECULAR STUDIES:

ELISA TEST (R&D IL-1ra Quantikine)

Standard Curve

pg/ml				Mean
1000	0.959	0.992	0.959	0.97
500	0.444	0.44	0.501	0.461667
250	0.214	0.209	0.225	0.216
125	0.089	0.094	0.097	0.093333
62.5	0.037	0.038	0.039	0.038
31.2	0.008	0.001	0.004	0.004333
15.6	-0.004	0.002	-0.004	-0.002
0	-0.009	0.018	-0.006	0.001



IL-1ra levels: 851.11 pg/mL

$p < 0.01$